Presence of bacteria in the endometrium and oviduct of cows with pyometra as detected by flourescence in situ hybridization

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Table 1. Pregnancies per embryo transfer (P/ET), crown-rump length (CRL), and pregnancy loss in embryo recipients receiving gonadotropin-releasing hormone (GnRH) on Day 5.5 *v.* control

Embryo stage	Group	P/ET Day 33, % (*n*)	P/ET Day 60, % (*n*)	Pregnancy loss, % (*n*)	CRL, mm ± SEM (*n*)
6	Control	28.6 (374)	22.7 (374)	20.6 (107)^{AC}	10.7 ± 0.3 (50)
	GnRH	30.8 (357)	23.5 (357)	23.6 (110)^{AC}	10.6 ± .2 (61)
7	Control	45.9 (394)	33.5 (394)	27.1 (181)^{BC}	10.8 ± 0.2 (84)
	GnRH	42.1 (437)	35.7 (437)	15.2 (184)^{A}	10.6 ± 0.2 (81)
P-value	Stage	<0.01	<0.01	0.45	0.98
	GnRH	0.90	0.35	0.16	0.46
	Interaction	0.30	0.67	0.04	0.33

^{A,B,C}Values with different superscript ($P < 0.01$).

Epidemiology/Diseases

111 RISK OF *CHLAMYDIA ABORTUS* TRANSMISSION VIA EMBRYO TRANSFER USING *IN VITRO* EARLY BOVINE EMBRYOS

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Chlamydia abortus (*C. abortus*) in cattle has been reported sporadically throughout the world and is implicated in respiratory, ocular, and reproductive disease as abortion, infertility, chronic mastitis, vaginal discharge, and endometritis. In addition, *C. abortus* presents a zoonotic risk exposure of pregnant women to infected animal and can lead to severe septicaemia in the mother, resulting in spontaneous abortion or stillbirth of the fetus. To investigate the risk of *C. abortus* transmission via bovine embryo transfer, our study aims to determine whether the embryonic ZP of *in vitro*-produced embryos protects early embryo cells against *C. abortus* infection and whether the bacteria adhere to or infect the cells of early bovine embryos (ZP-free) after *in vitro* infection. We also evaluated the efficacy of the washing procedure recommended by the IETS to decontaminate bovine embryos exposed to *C. abortus in vitro*. Ninety (8 to 16 cells) bovine embryos, produced *in vitro*, were randomly divided into 10 batches. Eight batches (4 ZP-intact and 4 ZP-free) of 10 embryos were incubated in a medium containing 4.8×10^7 Chlamydia/mL of AB7 strain (ANSES, Maisons-Alfort, France). After incubation for 18 h at 37°C in an atmosphere of 5% CO_2, the embryos were washed in batches in 10 successive baths of a PBS and 5% FCS solution without trypsin nor antibiotics in accordance with IETS guidelines. In parallel, 2 batches of 5 embryos (1 ZP-intact and 1 ZP-free) were subjected to similar procedures but without exposure to *C. abortus* as a control group. The 10 washing fluids from each batch were collected and centrifuged for 1 h at 13 000 × *g*. The embryos and wash pellets were tested using RT-PCR. *Chlamydia abortus* DNA was found in all ZP-intact and ZP-free infected embryos after 10 successive washes. It was also detected in the tenth wash fluid for 1 batch (1/4) of ZP-intact infected embryos and in 3 batches (3/4) of ZP-free infected embryos. In contrast, none of the embryos or their washing fluids in the control batches was DNA positive. These results demonstrate that *C. abortus* adheres to or penetrates the ZP as well as the early embryonic cells of *in vitro*-produced bovine embryos after *in vitro* infection, and that the standard washing protocol recommended by the IETS failed to remove it. The persistence of these bacteria after washing makes the embryo a potential means of transmission of the bacterium during embryo transfer from infected donor cows to healthy recipients or their offspring. Nevertheless, the finding of *C. abortus* DNA by RT-PCR did not imply that the bacteria found is still infective. Further studies are required to investigate whether enzymatic or antibiotic treatment of bovine embryos infected by *C. abortus* would eliminate the bacteria from the ZP.

112 PRESENCE OF BACTERIA IN THE ENDOMETRIUM AND OVIDUCT OF COWS WITH PYOMETRA AS DETECTED BY FLUORESCENCE *IN SITU* HYBRIDIZATION

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The objective of the study was to identify the location of the present bacteria in the uterus and oviducts of cows with pyometra. Pyometra is one of the postpartum infectious diseases in cattle that can result in infertility and thereby affect reproduction performance. Reproductive tracts ($n = 21$) were collected at a slaughterhouse in Denmark and send to The University of Copenhagen for examination and sampling. The uteri were included in the study when the following criteria were met: the cow was more than 21 days postpartum, the uterus was distended with pus, the cervix was closed, and a corpus luteum was present in one or both ovaries. A full thickness uterine tissue sample from the previous pregnant horn and both oviducts were sampled and then fixed in formalin. The tissues were trimmed, processed by routine methods, embedded in paraffin, sectioned at 3 microns, and prepared for fluorescence *in situ* hybridization using a probe targeting the 16S ribosomal RNA of the domain bacteria (i.e. targeting all bacteria regardless of species). Using fluorescence microscopy, the presence of bacteria within or on the surface of the endometrium and in the oviducts were noted. The endometrial biopsies from all cows ($n = 21$) contained bacteria, while 75% (16/21) of the cows had bacteria in one or both oviducts.

The bacteria were located on the luminal surface and in the lamina propria in 38.1% (8/21) of the uterine biopsies. In the remaining 62% of the uterine biopsies, the bacteria were only located above the basal membrane. Regarding the oviduct biopsies, the bacteria were located on the luminal surface and in lamina propria in 9.5% (2/21) of the biopsies, whereas the bacteria were located only above the basal membrane in 90.5% of the biopsies. In conclusion, 1) bacteria are present in the uteri and oviducts of cows with pyometra and 2) the bacteria are primarily located on the luminal epithelia surface above the basal membrane. Further analyses will investigate which specific species of bacteria that are located in the lamina propria of the uterine and oviduct biopsies.

113 EXPRESSION OF BETA-OXIDATION-RELATED GENES IN PREECLAMPSIA-LIKE MODEL UNDER HYPOXIC CONDITION *IN VIVO* AND *IN VITRO*

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Preeclampsia (PE) is a disorder of pregnancy characterized by high blood pressure and large amounts of protein in the urine. Preeclampsia is thought in many cases to be caused by a shallowly implanted placenta that becomes hypoxic. The hypoxic condition during the pregnancy can result from a failure at any stage in the delivery of oxygen to the cells. In peripheral tissues, oxygen diffuses down a pressure gradient into cells and moves into their mitochondria, where it is used to produce energy. As an expression of beta-oxidation-related genes, *ACADVL* was detected by gene-fishing technology using the placenta of human. We conducted *in vitro* and *in vivo* experiments to confirm preliminary study by inducing hypoxic stress in the BeWo cells and mice placenta. BeWo cells were cultured at 37°C under 1% O_2, 5% CO_2, and balanced with N_2. Pregnant mice were maintained from GD 6.5 to 17.5 under 11% O_2, 5% CO_2, and balanced with N_2. The expression of beta-oxidation related genes (*ACADVL*, *EHHADH*, *HADH*, *ACAA1*) were observed under hypoxic condition at mRNA and protein levels. The expression of genes known as biomarkers for hypoxia, *HIF-1α*, was increased in BeWo cells and mouse placenta, which induced PE. The beta-oxidation-related genes *ACADVL* expression was significantly increased by hypoxic stress both BeWo cells and mouse placenta. The elevated level of *HIF-1α* indicates that our experimental conditions closely mimicked PE. These results indicate that changes of beta-oxidation-related genes are correlated with PE induced hypoxic condition.

114 ENDOPLASMIC RETICULUM (ER) STRESS IN HYPOXIA-INDUCED DIABETES MELLITUS MODEL

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Endoplasmic reticulum (ER) regulates calcium ion concentration as a reservoir in the cell. ER stress is a cellular stress response related to the endoplasmic reticulum. At the initial stage of ER stress, ER tries to restore normal function by halting protein translation, degrading misfolded proteins, and increasing production of chaperones involved in protein folding. If ER fails to restore ER stress, ER stress can lead cells to apoptosis. To study the signaling between ER stress and calcium channels under ER-stressed circumstances, we designed a hypoxia-induced diabetic model. Nine-week-old male mice were chosen, maintained under hypoxic condition under 10% O_2, 5% CO_2 for 10 days, and the expression of ER stress markers and calcium channel gene expression were examined by real-time PCR. By maintaining hypoxic condition, the mice showed high glucose levels. Under this diabetic condition, in pancreatic beta cells, ER stress markers were elevated. This tendency showed an increase in calbindin-D_{9k} KO mice. Chaperones such as calreticulin and calnexin were decreased, but in calbindin-D_{9k} KO mice chaperone calnexin was not decreased. Interestingly, the calbindin-D_{9k} KO normoxia mice showed increased glucose level compared with wild-type normoxia mice. Also, calnexin expression of pancreas was decreased in calbindin-D_{9k} KO normoxia mice. This result indicates that pancreas cells were under endoplasmic reticulum stress. Taken together, calbindin may play an important role in endoplasmic reticulum stress in pancreas.

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Exotic Species

115 CHARACTERIZING NEUTROPHIL PROFILES IN HORSES FOR RHINOCEROS CAPTURE

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Conservation of several African species is becoming essential, and efforts to move threatened animals are causing physiological and reproductive problems. To save these species, a more comprehensive knowledge of their biology and response to stressors is required. Capture stress of rhinoceroses has been quantified (Kruger *et al.* 2011 Reprod. Fertil. Dev. **23**, 181–182) by evaluating leucocyte coping capacity (LCC). LCC is the measurement of the fluorescence of circulating active neutrophils, then expressed as optical density (OD)/1000 neutrophils. The LCC then provides a